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LABORATORY INVESTIGATION

Elimination of *Candida albicans* from kidneys of mice during short-term systemic infections

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Elimination of *Candida albicans* from kidneys of mice during short-term systemic infections. The candidacidal activity of kidneys, liver, and spleen's phagocytic systems was studied in mouse. Different strains of mice were inoculated intravenously (i.v.) with 1 to 2.6 × 10^4 viable *Candida albicans*. Elimination of the microorganisms from the kidneys, liver, and spleen were evaluated by enumeration of colony forming units (C.F.U.) recovered from homogenates of organs dissected within a short period of time (0 to 5 hr). The results indicated that the kidneys possess a capable phagocytic system which was able to eliminate the microorganisms as efficiently as those of liver and spleen. Furthermore, the ability of the liver and spleen phagocytic system as well as that of kidneys were significantly enhanced when animals were treated with Bacillus Calmette-Guerin (BCG) of mycobacterium bovis four weeks prior to induction of systemic infection with *C. albicans*.

Candida albicans remains one of the organisms most frequently producing infection in the compromised host [1, 2]. In the normal individuals, it has become apparent that a formidable barrier to *C. albicans* is provided by a multitude of effector and control mechanisms [3–16]. Yet, clearly defined experimental evidence for the precise mechanism(s) of resistance, either innate or acquired, to candidosis is lacking. Antibodies [15, 17, 18] and other serum factors [14, 19], T-lymphocytes [1–3, 8, 11, 13, 15, 16] and phagocytes [3, 4, 7, 10, 12, 13, 20] all have been implicated in resistance to Candida infection. However, the individual contribution(s) of each of these resistance factors is not well defined.

In the majority of experimentally-induced systemic infections in different conventional strains of mice, the kidneys of animals were the organ that bore the heaviest foci of infections throughout the experiments [3, 4, 6, 9, 11, 16]. By contrast, the liver and spleen, even in T cell deficient nude mice, were capable of clearing the candida infections [11, 16]. Recently, we reported that in the beige mouse, a murine model of human Chediak-Higashi syndrome (CHS), the liver and spleen of the animal were unable to clear the infection and remained infected throughout the trial [3, 4]. Since the beige mouse was shown to have a defective immune system, including abnormal polymorphonuclear leukocytes (PMNs) [21], we proposed an important role for these cells in fighting systemic candidosis. We further suggested that in the absence of normally functioning PMNs, activated macrophages play a decisive role in removing the microorganisms from the liver and spleen of the beige mouse [3]. It is unclear why kidneys are so much more susceptible to candida infections. Even in the BCG treated beige mouse, where the liver and spleen, due to activation of macrophages, were able to clear the candida infections, the kidneys still remained heavily infected [3].

It has been shown that mesangial cells have several macrophage activities. They exhibit phagocytic activities and produce interleukin-1 and lysosomal enzymes [22–25]. These macrophage-like properties make the mesangial cell a potential candidate to act as renal resident macrophage.

This study was initiated to investigate the early events of the experimentally induced systemic candidosis in terms of the role of phagocytes in clearing microorganisms during one to five hours post-infection, from kidneys, liver and spleen of different strains of mice.

Methods

Microorganism

Candida albicans strain B311 (type A), originally obtained from H.F. Hasenclever, National Institutes of Health (Bethesda, Maryland, USA), has been maintained in our laboratory by monthly transfers on Sabouraud dextrose agar (SDA) slants. Yeast cells were cultured for 24 hours at 37°C on SDA then stored at 4°C. For experiments, a single stock was prepared with organisms that were grown on SDA for 24 hours at 37°C, washed from the slants, and stored at −70°C in 1 to 2 ml of sterile saline at a concentration of 1 × 10^8 viable units per ml. The standard i.v. challenge used in this study was 1 × 10^4 viable units in 0.25 ml of phosphate-buffered saline (PBS) injected into a lateral tail vein.

The morphology and biochemical characteristics of *C. albicans* were verified by microscopic observations, colonial morphology on SDA, formation of germ tubes in serum, formation of chlamydospores on chlamydospore agar, and sugar fermentation reactions.

Mycobacterium bovis strain GL2 (BCG) was a gift from Jacqueline Vanderwinkel, Pasteur Institute, Brussels, Belgium, and was maintained at −70°C in 0.85% saline solution containing 0.1% gelatin [20]. Mice were injected i.v. with a single dose containing approximately 5 × 10^5 BCG in 0.25 ml saline four weeks before challenge with *C. albicans*. Experiments with *Listeria monocytogenes* (3 × 10^3, i.v.) served as the control to

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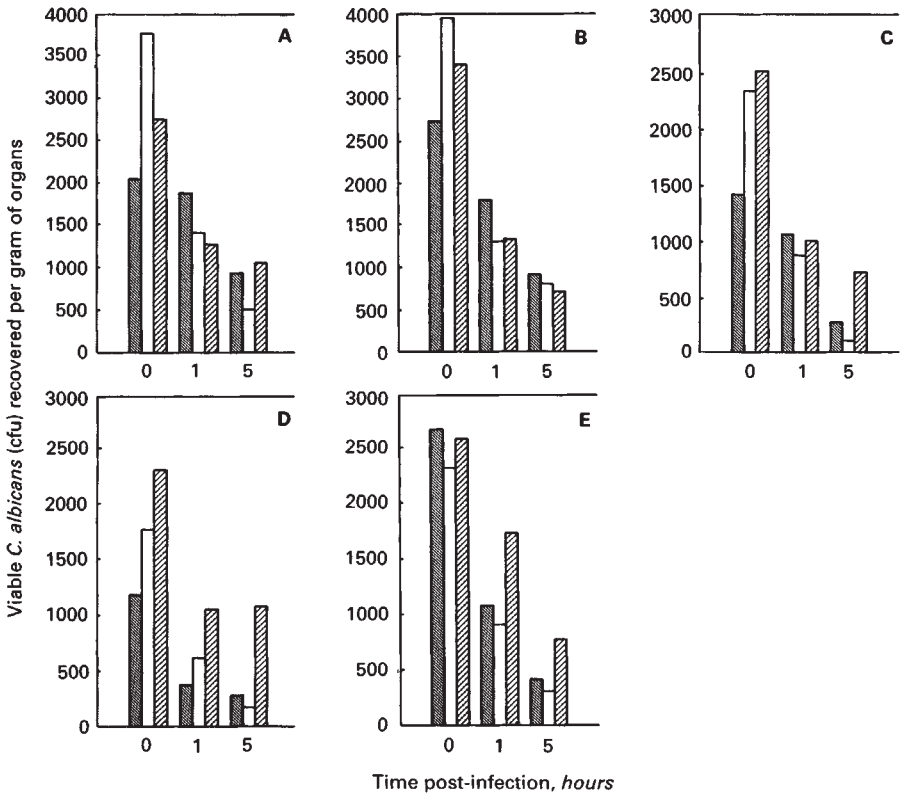

Fig. 1. *Recovery of C. albicans (cfu) from organs of different strains of mice following i.v. inoculation of 1 × 10⁴ viable organisms.* Symbols are: (▩) livers; (□) kidneys; (▨) spleens. The results report the number of cfu obtained by pooling corresponding organs from 3 to 5 mice. **(A)** Beige mouse; **(B)** Beige normal littermate; **(C)** BALB/c mouse; **(D)** BALB/c nude mouse; **(E)** Swiss Webster mouse.

substantiate that our BCG treatment protocol resulted in enhanced macrophage function. The number of *L. monocytogenes* cultured from the spleens and livers of BCG-treated mice was 1,000- to 10,000-fold less than cultured from the respective organs of PBS-treated control mice.

L. monocytogenes was provided by Dr. Edward Balish, Department of Surgery, University of Wisconsin, Madison. The organism was grown on 5% sheep blood agar at 37°C for 48 hours, collected in saline, and washed by centrifugation. Organisms were resuspended in the gelatin solution as described above and frozen at −70°C [20].

Mice

Congenitally athymic (nude) BALB/c mice and their thymus-bearing phenotypically NLMs were produced by mating homozygous (nu/nu) males with heterozygous (nu/+) females. Nude and nu/+ mice were bred and housed in Bioclean Porta-Room containment units (Hazleton Systems, Inc., Aberdeen, Maryland, USA) and were offered food and acidified-chlorinated water ad libitum. The lack of a thymus was confirmed in each mouse at necropsy. Groups of nude mice were periodically assessed for their ability to reject allogenic or xenogeneic skin grafts and to form antibodies to a thymus-dependent antigen, sheep erythrocytes.

Beige mice and their phenotypically NLMs (bg/+) on a C57BL/6 background were produced by mating the appropriate homozygous (bg/bg) and heterozygous (bg/+) animals. C57BL/6 mice were maintained under conventional housing conditions and were offered food and water ad libitum. Swiss-Webster mice were produced by random mating and maintained as were C57BL/6 mice.

Calculation of candidacidal activities of phagocytic systems of the organs

Percent killing of *C. albicans* by phagocytes was calculated as follows:

$$\text{Percent killing} = \frac{\text{cfu recovered at ''0'' hour} - \text{cfu recovered at a point of time} \times 100}{\text{cfu recovered at ''0'' hour}}$$

Results

Recovery of C. albicans from kidneys, liver, and spleen of different strains of mice during short-term systemic candidosis

Results presented in Figures 1 and 2 display cfu recovered from the kidneys, livers, and spleens of different strains of mice following intravenous inoculation with 1 × 10^4 viable organisms. Results of these two separate experiments revealed that the three organs of all strains of mice cleared a significant number of the inoculated organisms during five hours of infection. The phagocytic system of kidneys, in particular, showed distinct efficiency in eliminating *C. albicans* during this short period (Figs. 1 and 2).

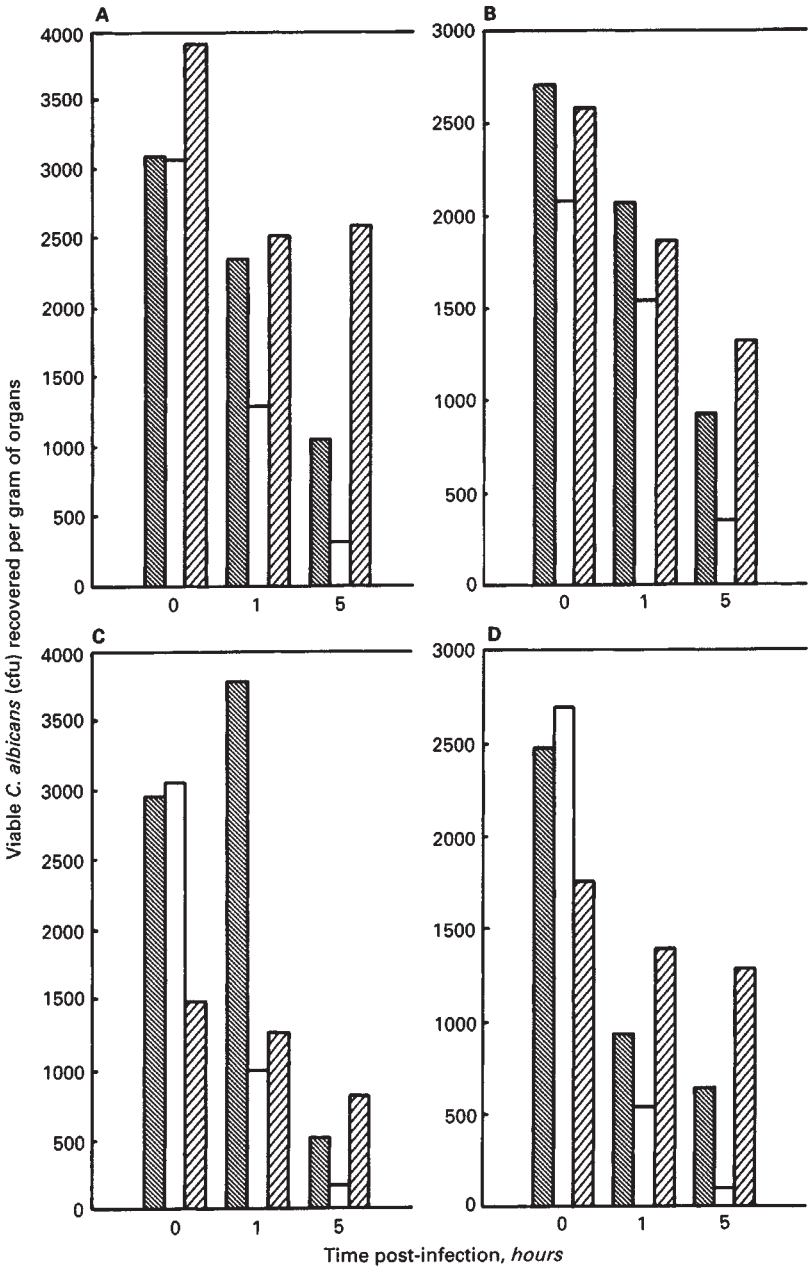

Fig. 2. *Recovery of C. albicans (cfu) from organs of different strains of mice following i.v. inoculation of 1 × 10^4 viable organisms.* Symbols are: (▩) livers; (□) kidneys; (▨) spleens. The results report the cfu obtained by pooling corresponding organs from 3 to 5 mice. **(A)** Beige mouse; **(B)** Beige normal littermate; **(C)** BALB/c mouse; **(D)** BALB/c nude mouse.

Recovery of C. albicans *from kidneys, liver, and spleen of animals treated with BCG*

In experiments 3 and 4, when PBS and BCG treated mice were intravenously inoculated with 1 × 10^4 (Fig. 3) and 2.6 × 10^4 (Fig. 4) viable *C. albicans* the three organs of different strains of mice cleared a significant number of the organisms during a five hours period of infection. The phagocytic system of PBS treated mice demonstrated activity similar to the observations obtained from experiments 1 and 2 (Figs. 1 and 2) in clearing the organism from these organs. However, phagocytic systems of the organs of animals treated with BCG showed more candidacidal activities than those of PBS treated animals. This activity was more obvious in the kidneys and spleen at five hours post-infection (Figs. 3 and 4). This ability was uncompromised when the number of inoculated organisms was increased over 2.5-fold (Fig. 4).

Percent killing by phagocytic systems of kidneys, liver, and spleen

Tables 1 to 4 demonstrate the percent killing of *C. albicans*, by the phagocytic systems of these organs. The percent of killing were calculated from the results of these four experiments.

Discussion

Renal burden of *C. albicans* during systemically-induced candida infections in all strains of mice studied remained high [3, 4, 6, 9, 11, 16]. It has been suggested that the physiological

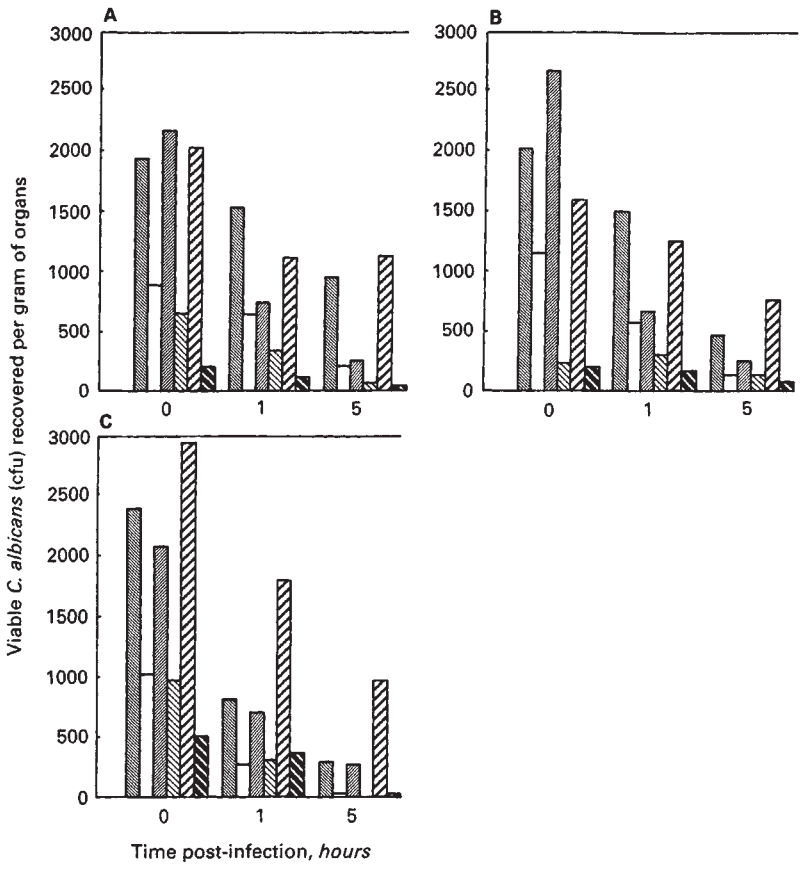

Fig. 3. *Recovery of C. albicans (cfu) from organs of different strains of mice treated with PBS or BCG.* Mice were inoculated i.v. with 1×10^4 viable organisms four weeks after BCG treatment. Symbols are: (▨) livers PBS; (□) livers BCG; (▨) kidneys PBS; (▨) kidneys BCG; (▨) spleens PBS; (▨) spleens BCG. The results report the cfu obtained by pooling corresponding organs from 3 to 5 mice. **(A)** Beige mouse; **(B)** Beige normal littermate; **(C)** Swiss-Webster mouse.

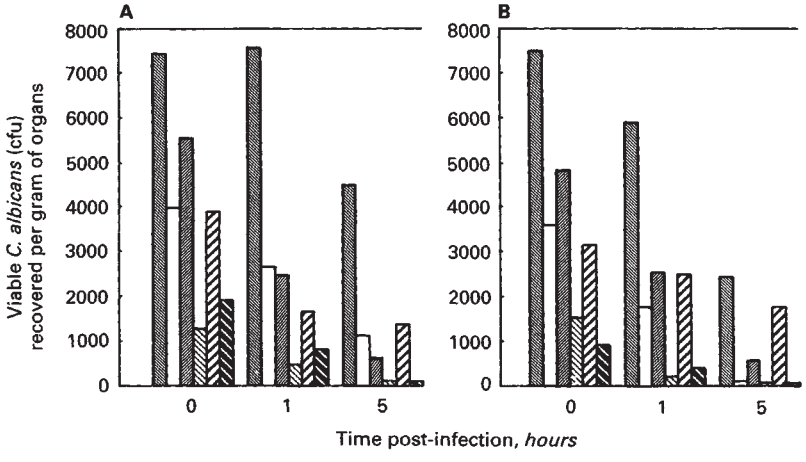

Fig. 4. *Recovery of C. albicans (cfu) from organs of mice treated with PBS and BCG.* Symbols are: (▨) livers PBS; (□) livers BCG; (▨) kidneys PBS; (▨) kidneys BCG; (▨) spleens PBS; (▨) spleens BCG. Mice were inoculated i.v. with 2.6×10^4 viable organisms four weeks after BCG treatment. Each number is the cfu obtained by pooling corresponding organs from 3 to 5 mice. **(A)** Beige mouse; **(B)** Beige normal littermate.

conditions [26–28] and the poor phagocytic system of kidneys [29] are contributing factors for predisposition of kidneys to the long-term systemic candida infections.

Based on our observations and those of others, we suggest that perhaps anatomical architecture and physiological conditions of kidneys contribute to their susceptibility to candida infections. We assume that a few yeasts may escape the renal phagocytic system and reside in regions such as the renal medulla or tubular area [26, 28] where, due to increased osmolarity and high content of urea and ammonia, the phagocytic and chemotactic activities of recruited PMNs and monocytes are reduced [29, 27]. The delay in inflammatory response reported by Louria, Brayton and Finkel [26] certainly helps the yeasts develop pseudohyphae, penetrate the tubular area [26, 28] and produce foci of infection.

It has been shown that pretreatment of mice with BCG or its analogues induces activation of macrophages [30, 31]. We presumed that if elimination of *C. albicans* from organs of PBS-treated mice is mainly due to activities of phagocytic systems therefore, treatment of animals with BCG prior to

Table 1. Candidacidal activities (percent)

	Hours post-infection					
	Kidneys		Liver		Spleen	
Mouse strains	1	5	1	5	1	5
Beige	62	86	7	54	53	62
Beige NLM	66	79	33	66	61	78
BALB/c	62	94	25	79	60	71
BALB/c nude	65	89	68	75	54	53
Swiss-Webster	60	86	59	84	32	69

Percent candidacidal activity of phagocytes calculated from results presented in Figure 1.

Table 2. Candidacidal activities (percent)

	Hours post-infection					
	Kidneys		Liver		Spleen	
Mouse strains	1	5	1	5	1	5
Beige	58	59	24	66	35	34
Beige NLM	25	83	23	66	28	49
BALB/c	67	94	0	82	15	45
BALB/c nude	80	96	62	74	21	26

Percent candidacidal activity of phagocytes calculated from results presented in Figure 2.

candida infection should enhance these activities. The results presented in Figures 3 and 4 as well as Tables 3 and 4 indicated that this is the case. Treatment of the animals with the BCG enhanced clearing capabilities of the three organs significantly. Therefore, we suggest the enhanced candidacidal activities of the organs from BCG-treated animals within this short period of time is due to activation of phagocytic systems. These results also argue against a possible role of physiological clearing of microorganisms through endocytosis or renal lymphatics. In fact, the number of organisms recovered from organs of BCG treated animals at time "0" was far below the number of organisms recovered from corresponding organs of control animals treated with PBS. This discrepancy is due to 40 to 60 minutes processing steps during which organs had to be removed and weighed before being homogenized and plated. It was during this period that activated macrophages with increased bactericidal capabilities acted more efficiently than macrophages of PBS-treated animals. For this reason the percent killing activities of BCG-treated animals were also calculated by using cfu recovered from the organs of PBS treated animals at "0" hours (shown in parentheses, Tables 3 and 4). The percent of killing presented in parentheses is a more accurate representative of candidacidal activities of phagocytes of the organs from BCG-treated animals. Activation of the phagocytic systems by BCG was more obvious when treated animals were inoculated with an over 2.5-fold higher number viable *C. albicans* (Fig. 4, Table 4).

We should also mention that hepatic and splenic predisposition of beige mice to candida infection reported previously [3, 4] is an effect which is observed in the long-term infections. The results of short-term candida infections do not show such an inclination. Reasons for this discrepancy are not yet clear. However, we suggest that hepatic and splenic predisposition to long-term systemic candida infections in beige mice could be

Table 3. Candidacidal activities (percent)

	Hours post-infection					
	Kidneys		Liver		Spleen	
Mouse strains	1	5	1	5	1	5
Beige (PBS)	65	88	21	50	45	44
Beige (BCG)	47 (84)	88 (96)	27 (67)	75 (89)	38 (93)	74 (97)
Beige NLM (PBS)	75	91	26	77	21	53
Beige NLM (BCG)	0 (89)	44 (95)	50 (72)	89 (94)	17 (89)	63 (95)
Swiss-Webster (PBS)	65	86	65	87	39	67
Swiss-Webster (BCG)	65 (84)	98 (99)	73 (88)	96 (98)	27 (87)	92 (99)

Percent candidacidal activity of phagocytes calculated from results presented in Figure 3. Numbers in the parentheses are representatives of killing activities of organs of BCG-treated animals when cfu recovered from corresponding organs of PBS-treated animals at "0" hours for corresponding strain of mouse is taken into the account.

Table 4. Candidacidal activities (percent)

	Hours post-infection					
	Kidneys		Liver		Spleen	
Mouse strains	1	5	1	5	1	5
Beige (PBS)	55	88	0	39	57	64
Beige (BCG)	62 (91)	89 (97)	32 (64)	71 (84)	56 (79)	93 (96)
Beige NLM (PBS)	47	88	21	67	20	43
Beige NLM (BCG)	85 (95)	95 (98)	51 (76)	96 (98)	56 (87)	92 (98)

Percent candidacidal activity of phagocytes calculated from results presented in Figure 4. Numbers in parentheses are representatives of killing activities of organs of BCG-treated animals when cfu recovered from corresponding organs of PBS-treated animals at "0" hours for corresponding strain of mouse is taken into the account.

due to recruitment of premature monocytes and PMNs from bone marrow. These cells contain greater numbers of abnormal giant lysosomes [32] which are defective in phagosome-lysosome fusion process [32]. This step is an essential phase in bactericidal activities of phagocytes. Therefore, when these abnormal phagocytes are recruited to the area of infection they are unable to combat the microorganism effectively. This situation provides an opportunity for the yeast to grow and produce infectious foci in liver and spleen.

Early investigators used the nude mouse in systemically induced candida infections, and they have questioned the involvement and importance of CMI in systemic candidosis [6, 11, 16]. Nude mouse organs cleared the candida infections during the short-term systemic infections as efficient as those of their normal litter mates. These results support the previous findings.

Our results demonstrated that in contrast to previous conclusions, kidneys possess a strong phagocytic system, and BCG treatment of animals potentiates this system. Our result also suggested that the phagocytic system is a crucial defense line against systemically-induced candida infections.

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